Experimental in-stent restenosis in rats

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Summary and discussion

This thesis describes: 1) the development of a new and reliable animal model for in-stent restenosis (ISR), which enables thorough pathophysiological stent research and the screening of anti-restenotic therapies, 2) the necessity for additional physiological anti-restenotic strategies and 3) the evaluation of endothelial function improvement, as a method for more physiological anti-restenotic therapy.

In ***Chapter 2***, elaborate descriptions of the pathophysiological process of ISR and the role of the renin-angiotensin system (RAS) in ISR are provided. Restenosis is the arterial healing process as seen after vascular injury inflicted during percutaneous transluminal coronary angioplasty (PTCA). Restenosis after balloon angioplasty is caused by elastic recoil, negative remodelling and neointimal formation. Stent implantation prohibits virtually all elastic recoil and negative remodelling after PTCA. However, restenosis still occurs, now mainly due to excessive neointimal formation. Neointimal formation ensues from endothelial denudation and deep vascular injury, which result in subsequent thrombus formation, inflammation and smooth muscle cell proliferation.
ISR is currently effectively prevented by drug-eluting stents (DES). These stents coated with strong anti-proliferative agents potently inhibit smooth muscle cell proliferation and consequently ISR. However, due to findings such as incomplete re-endothelialisation, delayed vascular healing, late thrombosis and hypersensitivity reactions after DES implantation, major concerns with regard to the late effects of these stents have emerged. Therefore, there is an increasing interest for more physiological anti-restenotic strategies.
The systemic use of RAS intervention in cardiovascular disease is widespread and rather safe. The RAS plays a role in the pathophysiology of ISR. RAS intervention by means of angiotensin-converting enzyme (ACE) inhibition, angiotensin II type 1 (AT_1) receptor blockade or angiotensin-(1-7) (Ang-(1-7)) might be an appealing method for prevention of ISR in a physiological manner.
The use of ACE inhibitors as anti-restenotic therapy is disputable. ACE inhibitors are probably ineffective due to the need for high dosing to inhibit tissue ACE and alternative angiotensin II forming pathways. The effects of alternative angiotensin II formation can be avoided by means of AT_1 receptor blockade. AT_1 receptor blockers have been proven to be effective in the prevention of ISR in animal models and in humans. Combination of an ACE inhibitor and an AT_1 receptor blocker might be even more effective, as a consequence of additional bradykinin-dependent nitric oxide formation. Recently, Ang-(1-7) has also been shown effective in preventing restenosis after vascular injury and stent implantation. Future experiments concerning RAS intervention to prevent ISR in a physiological manner, should focus on the local application of AT_1 receptor blockers, combination therapy and Ang-(1-7), by means of DES.

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Rat abdominal aorta stenting model

Chapter 3 describes the development of a new animal model of ISR. Coronary stents were implanted in the abdominal aorta of the rat. After 1, 3, 7, 28 and 56 days the rats were sacrificed and the pathophysiological mechanisms of ISR were evaluated. Furthermore, a known anti-restenotic stent was implanted to examine the predictive value of this model. Surgical procedures were generally well tolerated by the animals. Neointimal measurements such as, neointimal area, neointimal thickness and percentage stenosis increased up to 28 days after stent placement. Thereafter, a slight decrease in neointimal parameters was observed. Moreover, strong positive linear correlations were observed between the mean injury score and neointimal measurements. These patterns of neointimal formation are similar as seen in the well-established pig coronary and the rabbit iliac artery ISR models.

As for pathophysiological mechanisms of ISR, we observed focal thrombus formation in the early stages of this model. Adhesion and infiltration of leukocytes occurred as soon as 1 day after stenting and they peaked after 3 and 7 days, respectively. The inflammatory response was virtually absent after 28 days. In the late stages of this model a clear neointima was present, which consisted of smooth muscle-like cells and extracellular matrix. These vascular responses develop similar as in other ISR models.

Finally, we found a reduction in neointimal formation after implantation of a known anti-restenotic stent. Thus, the rat abdominal aorta stenting model is feasible, it displays similar vascular responses to stent implantation as seen in the established pig and rabbit ISR models and it is useful for testing anti-restenotic agents and intra-vascular devices.

Preclinical animal models of ISR are important but imperfect standards. Presently available ISR models are the pig coronary artery and rabbit iliac artery stent models. These models have some drawbacks. A combination of radiological and surgical utilities is required, most animal facilities have limited housing capacity for pig and rabbits and the costs for purchase are high. A simple, inexpensive, rapid and accurate preclinical model would be useful [1]. In the rat abdominal aorta stenting model only a surgical microscope and mainstream surgical equipment are required. The housing capacity for rat is less limited and the costs for purchase are low. The applicability of the rat model to test anti-restenotic therapies was shown by the effective inhibition of neointimal formation by rapamycin-eluting stents. Thus the rat abdominal aorta stenting model is a simple, inexpensive, rapid and accurate preclinical model for ISR.

Insights in pathophysiological mechanisms of ISR are essential for the development of new anti-restenotic therapies. Since only a limited number of antibodies to cellular proteins and transgenic and knockout strains are available, possibilities to perform pathophysiological experiments in the pig and rabbit models are restricted. Many antibodies as well as transgenic diabetic and hypertensive

strains are available in rats. Moreover, knockout strains are becoming available [2]. Consequently, the rat abdominal aorta stenting model enables thorough pathophysiological research.
Therefore, the rat abdominal aorta stenting model is a simple, inexpensive, rapid and accurate preclinical model for in-stent restenosis, which enables thorough pathophysiological stent research and the screening of anti-restenotic therapies.

Drug-eluting stents and the necessity of physiological treatment
The effects of paclitaxel-eluting TAXUS stent implantation in absence of anti-platelet therapy are specified in ***Chapter 4***. In the absence of anti-platelet therapy, paclitaxel-eluting stent placement resulted in signs of delayed healing, such as fibrin deposition and intra-intima hemorrhage. As expected, paclitaxel-eluting stent implantation resulted in a decrement in neointimal cell density. Furthermore, measurements of neointimal area showed an increase after paclitaxel-eluting stent implantation as compared to bare metal stent placement. This emphasises the necessity of anti-platelet therapy after implantation of the present generation DES.

Presently, ISR is effectively controlled in clinical practice by DES. Stents coated with paclitaxel strongly inhibit smooth muscle cell proliferation and subsequent neointimal formation [3,4]. Nevertheless, paclitaxel-eluting stents have potential long-term adverse sequelae. Recent studies suggest that paclitaxel-eluting stent show incomplete re-endothelialisation, late stent thrombosis and delayed vascular healing [5-9]. These findings of delayed healing after paclitaxel-eluting stent implantation are confirmed by our results.
Delayed re-endothelialisation may lead to increased susceptibility for late thrombosis after discontinuation of anti-platelet therapy. Furthermore, in the absence of anti-platelet therapy delayed re-endothelialisation after paclitaxel-eluting stent implantation may result in excessive adhesion of platelets and inflammatory cells to the injured vascular wall, with subsequent increased neointimal formation. Delayed re-endothelialisation may explain why paclitaxel-eluting stent placement in the absence of anti-platelet therapy results in exaggerated neointimal formation. This emphasises the importance of anti-platelet therapy after paclitaxel-eluting stent implantation. More importantly, incomplete re-endothelialisation after stenting is precarious. This underlines the necessity of optimisation of current anti-restenotic strategies and development of additional more physiological anti-restenotic strategies.

Recovery of systemic endothelial function and ISR
In ***Chapters 5, 6 and 7*** the influence of improvement of systemic endothelial function on ISR is illustrated. ***Chapter 5*** describes that stent implantation in the rat abdominal aorta resulted in a substantial deterioration of systemic endothelial function. Intravenous Ang-(1-7) treatment results in an almost complete recovery

of systemic endothelial function. Moreover, Ang-(1-7) attenuated neointimal formation after stenting in the rat abdominal aorta. In ***Chapter 6*** the long-acting erythropoietin (EPO) analogue, darbepoetin also was shown to improve systemic endothelial function. However, with this drug, recovery of endothelial function was not accompanied by a reduction in the volume of neointimal tissue growth. A detailed description of the relationship between systemic endothelial function and neointimal formation is provided in ***Chapter 7***. The effect of stenting on systemic endothelial function and its different mediators was examined. Concurrently, the effects of Ang-(1-7) and darbepoetin treatment were studied. Finally, correlations between systemic endothelial function were determined. Stent placement resulted in a strong impairment of systemic endothelial function. This impairment was characterised by a major reduction in nitric oxide (NO)-dependent vasodilation, which was partly compensated by an increase in endothelial derived hyperpolarizing factor (EDHF)-dependent vasorelaxation. The Ang-(1-7) induced recovery of systemic endothelial function was brought about by an increase in prostaglandin (PG)- and EDHF-dependent vasodilation. darbepoetin also improved systemic endothelial function after stent placement. However, this increase in endothelium-dependent vasorelaxation, was mainly due to a recovery of NO-dependent vasodilation. As darbepoetin-dependent recovery of systemic endothelial function was not accompanied by a reduction in neointimal formation, a relationship between systemic endothelial function and ISR seems unlikely. To strengthen this observation, correlations between systemic endothelial function and neointimal measurements were also absent.

Stent implantation results in severe endothelial disruption [10,11]. Stenting also induces pronounced endothelial dysfunction [11]. Moreover, a decreased systemic endothelial function is associated with the occurrence of ISR [12]. Improvement of the systemic endothelial function results in release of potential anti-restenotic substances, like NO, PG and EDHF. Thus, systemic endothelial enhancement might reduce ISR. Ang-(1-7) attenuates neointimal formation accompanied with a recovery of systemic endothelial function. Contrarily, darbepoetin also improves systemic endothelial function, however it does not influence neointimal formation. This taken together with the absence of correlations between endothelial function and neointimal measurements implies that there is no direct relation between systemic endothelial function and ISR. Therefore, experimental anti-restenotic strategies should restore the endothelium and its function, and in addition smooth muscle cell proliferation should be inhibited.

Future directions

For the development of future anti-restenotic regimes, elucidation of pathophysiological mechanisms of ISR is indispensable. The rat abdominal aorta stenting model enables thorough pathophysiological experiments. The usefulness of this model for explaining pathophysiological processes is already demonstrated by

Zhou *et al* [13]. Considering that this rat model is simple, rapid, inexpensive and accurate, the application of this model can become widespread. Thus, the rat abdominal aorta stenting model might make a substantial contribution to insights into pathophysiological processes of ISR and the development of future anti-restenotic therapies.

As for the currently available anti-restenotic strategies, the rapamycin- and paclitaxel-eluting stents, their long-term safety profile has to be determined. Presently, reports of incomplete re-endothelialisation, late thrombosis and delayed vascular healing emphasise the necessity for the development of more physiological anti-restenotic therapies. Physiological therapies should aim to act through the normal arterial healing process.

Improvement of systemic endothelial function may be an attractive alternative physiological anti-restenotic therapy. However, we demonstrated that improvement of the systemic endothelial function alone is not sufficient to inhibit neointimal formation. Previously, it was shown that promotion of local re-endothelialisation by vascular endothelial growth factor (VEGF) inhibits neointimal formation [14,15]. The latter observation suggests that promotion of re-endothelialisation is effective for prevention of ISR. Moreover, it suggests that there is dissociation between systemic endothelial function and re-endothelialisation, since systemic endothelial function improvement does not prevent neointimal formation. However, VEGF also appears to have a direct inhibitory effect on vascular smooth muscle cells [16]. Thus the problem whether re-endothelialisation alone is sufficient to inhibit ISR, remains unresolved. Stents coated with antibodies to CD34 receptors on circulating endothelial cells have been developed [17]. The results of experiments with these stents will give us more insight in this important controversy.

As for potential future physiological anti-restenotic substances, Ang-(1-7) is an attractive option. Firstly, Ang-(1-7) has beneficial effects on the endothelium [18,19]. Secondly, Ang-(1-7) inhibits smooth muscle cell proliferation [20]. Moreover, we have shown that systemic treatment with Ang-(1-7) attenuates neointimal formation. As systemic Ang-(1-7) treatment is no option, an Ang-(1-7)-eluting stent is a potential future solution for the problem of ISR.

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